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TITLE OF THE INVENTION (280 characters max)

ELECTRONIC DEVICE FOR COMMUNICATION WITH LIVING CELLS

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ENCLOSED APPLICATION PARTS (check all that apply)

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Respectfully submitted,

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ELECTRONIC DEVICE FOR COMMUNICATION WITH LIVING CELLS

FIELD OF THE INVENTION

This invention is generally in the field of bio-molecular electronics, and relates to an electronic device for communication with living cells.

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BACKGROUND OF THE INVENTION

Interaction between neurons and electronic devices has been in existence for several decades for a plurality of purposes. During the past decades, these interactions were usually achieved by inserting an electrode assembly (single electrode or an array of electrodes) into the neurons, or placing an electrode assembly in the vicinity of the neurons' membranes, so as to detect voltage changes. The detection electrode assembly can also be used for the stimulation of neurons.

Attempts have been made to provide coupling between a neuron and a transistor device [1], utilizing a bi-directional interface between the ionic conduction of the neuron and the electronic conduction of the silicon, achieved by two separate modalities.

WO 00/51191 (having the same inventors as in the present application) discloses an electrical junction between a transistor and a neuron, transistors to be used in said junction, and an artificial chemical synapse. "Chemical synapse" is a junction between a cell, which secretes an agent, and a transistor bearing receptors for the agent, wherein binding of the agent to the receptor changes an electrical property off the transistor.

SUMMARY OF THE INVENTION

The present invention provides a novel approach for implementing reliable and durable bi-directional electrical as well as chemical communication (functional linking) between neurons (or other cell types) and electronic devices. This generic technology can serve as the base for the construction of biomedical devices that can, for example, be used to functionally link nerves to robotic prosthesis and thus provide amputees with robotic prosthesis that are controlled by the brain, functionally link damaged neuronal networks, restore vision after retinal or optic nerve damage, etc. It can also be used to screen drugs or for the development of exvivo hybrids between electronic devices, cells and molecules.

The inventors have analyzed parameters and mechanisms that control the degree of coupling between biological cells such as neurons and electronic devices, identified critical unforeseen basic scientific and technological problems that must be solved in order to advance and fully implement the technology of neuro–electronic hybrid devices, and provide technical solutions for these problems.

The inventors have harnessed the basic properties of cells to identify, preferentially adhere and then to internalize elements of the extracellular world by phagocytosis (the term *phagocytosis* may interchangeably be used with the terms *pinocytosis* or *endocytosis*), and found that by making the gate electrode of a transistor with protrusions, these basic intrinsic properties of cells lead to the internalization of these protrusions, thereby providing optimal coupling between the transistor and the cell. The basic properties of biological cells, together with a novel design of the electrode surface, as well as tailored surface chemistry, provide for reducing the cell's mobility and producing tight physical linkage between cells and the surfaces of electronic or chemical devices and thereby producing tight electrical and chemical coupling.

As mentioned above, the electrode surface morphology is designed in a novel, rough form, consisting of protrusions projecting from the electrode surface. These protrusions are also termed hereinafter "micronails", or "nails" since they comprise a rod- or stem-like "base" portion and may also comprise a "head" portion, and their length is preferably ranging between tens of nanometers to thousands of nanometers. The diameters of the nails range between tens to hundreds of nanometers. The structure, dimensions and density of the micronails can be optimized to maximize the electrical and chemical coupling between the hybrid components, namely the transistor and the living cell.

The internalization of the micronails provide three major advantages: anchoring of the cell to the transistor thus reducing cell's motility; improved electrical coupling of neurons to the transistors by increasing the coupling capacitance and the increase of the resistance between the body of the biological solution and the coupling area; and improved chemical sensing (i.e., the formation

of artificial "chemical synapses"). Such a novel design and fabrication of the surface morphology of a gate electrode (the protrusion of micronails) is complimented by molecularly modified surfaces that decorate differentially the different parts of the nails.

As for the chemically modified surfaces, they induce such main cellular events as: assembly of adherence junctions and internalization of the micronails' heads by membrane invagination. This can be achieved by the following ways:

- The nail's head carries enzymes that facilitate degradation of the extracellular matrix of the cell and thus allow the formation of intimate physical contact between the surface of the micronail's head and receptor molecules located on the external surface of the plasma membrane. These enzymes are designed to be of short lifetime.

- The nail's head also carry molecules that recognize plasma membrane receptors, bind to these receptors and facilitate phagocytosis or endocytosis of the micronails' head by partial internalization of the micronails by the cells.

- The nail's base carries adhesion molecules that stabilize the binding of the cell's plasma membrane to the micronail's base and its surroundings.

The present invention provides a neurotransmitter sensing element, based on the formation of chemical synapse composed of a neuronal presynaptic element and an electronic device that serves as a post-synaptic element. The formation of such synapses enables to link neurons to the electronic device not only by electrical signals but also by released neurotransmitters. Such chemical linkage open up a novel way to link the nervous system with the electronic world and allow simulating the natural way by which neurons as well as muscles communicate with each other. It should be emphasized in this respect the unidirectional communication between excitable cells is mainly executed by chemical synapses. To this end, the micronails containing surface of a gate electrode is coated with a variety of signaling molecules and receptor molecules that recognize and bind acetylcholine, glutamate, GABA, serotonin and others.

The inventors have developed such three major components of the hybrids as electronic component, surface chemistry, and cell and neurobiology.

The electronic component is based on a transistor structure (preferably floating gate transistor as disclosed in the above-indicated publication WO 00/51191 assigned to the assignee of the present application), where the gate electrode has a special surface, which includes various types of protruding micronails. "Floating gate" is an insulated electrode of a MOS transistor on which an electric field is applied, thereby inducing an electric field to the active component of the transistor through capacitive coupling.

The surface chemistry uses advance technology to differentially link to the micronails, molecules that induce two main cellular events: assembly of adherence junctions and internalization of the micronails heads by membrane invagination. These events are facilitated in the following ways: (a) The nail head is linked to hydrolytic enzymes that facilitate degradation of the extracellular matrix and thus allow the formation of intimate physical contact between the micronails head's surface and receptor molecules located on the external surface of the plasma membrane; (b) The nail head also carries molecules that recognize plasma membrane receptors tether the micronail's head and facilitate phagocytosis or endocytosis of it, i.e., partial internalization of micronails by the plasma membrane; (c) The nail base carries adhesion molecules that stabilize the binding of the cell's plasma membrane to the nails' base and its surroundings.

The cell and neurobiology aspect of the invention consists of optimizing the conditions and chemical surfaces that lead to the differentiation of adhesion junctions, tethering and phagocytosis of gold particles.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is a schematic illustration of a neuroelectronic hybrid system;

Fig. 2 illustrates a scheme defining the geometrical relations between a cultured neuron and the transistor gate, and a seal resistance between the body of the biological solution and the neuron-transistor coupling area;

Figs. 3A and 3B schematically illustrate the main principles of the phagocytosis phenomenon, by showing schematic presentations of the cellular mechanisms that underlie phagocytosis (Fig. 3A), and its comparison to the engulfment of a micronail (Fig. 3B);

Figs. 4A-4D illustrate electron micrographs of endocytotic/phagocytotic profiles induced by the exposure of cultured *Aplysia* neurons to fragments of other cells, wherein Fig. 4A corresponds to the induction of endocytosis; Fig. 4B corresponds to the induction of an endocytotic profile by a particle; Fig. 4C corresponds to the endocytotic profile; and Fig. 4D corresponds to a coated vesicle containing a cell fragment;

Figs. 5 and 6 illustrate an electronic device (transistor) according to the invention utilizing a gate electrode formed with micronails which are protruding from gate electrode surface, wherein Fig. 5 shows an over view of the micronails configuration on the floating gate, and Fig. 6 shows the scaled cross-section of the 'nail' structure;

Fig. 7 illustrates two types of the Floating Gate Depletion-type Transistor (FGDT) based micronails sensors: *Type A* devices designed to enhance contact with neurons by applying various surface chemistries that facilitate wrapping of the nails by the cell's membrane, and *Type B* devices presenting a modification of the *type A* devices and being tailored to enhance sensitivity to molecular recognition events and chemical processes, with or without cells presence;

Fig. 8 illustrates a process of adhering hydrolytic enzymes to the nails surface, showing the micronails' head assembled with phagocytosis signaling peptides (PSP) and hydrolytic enzymes (HE) connected via biodegradable spacer (PLA/PGA);

Fig. 9 shows the degradation of the extracellular matrix and partial phagocytosis of the nails by plasma membrane invagination;

Fig. 10 exemplifies the technique of peptide anchoring to the nitride regions and to the gold nail-heads containing chromophoric layer, in *type A* devices, while *type B* devices will couple alkylhalide functionality to the oxide in step *i*, followed by steps *ii-iv* of this figure;

Fig. 11 exemplifies saccharides anchoring to the nitride regions and to the gold nail-heads containing chromophoric layer, in *type A* devices, while *type B* devices will couple alkylhalide functionality to the oxide in step *i*, followed by steps *ii-iv* of this figure;

Fig. 12 illustrates the synthetic route developed for the chemical/enzymatic self-assembly of 2D-PAN monolayer, including AFM images of the PSS template layer (left) and the2D-PAN (right) obtained via chemical oxidation;

Fig. 13 presents electron microscopy image showing the interface between Aplysia's plasma membrane and a monolayer of 5nm Au particles self-assembled with 2D-PAN monolayer on a glass substrate; and

Fig. 14 exemplifies neurotransmitter site containing proteins (enzyme/receptor, E/R) anchoring to gold surfaces via rigid and short spacer (*type A*).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is aimed at optimizing electrical and chemical coupling between neurons (generally, living cells) and a Field Effect Transistor (FET). The latter may, for example, be a floating gate depletion type transistor as disclosed in the above-indicated publication WO 00/51191 assigned to the assignee of the present application. "Depletion type device" is an insulated-gate field-effect transistor in which free carriers are present in the channel (active component) when the gate-source voltage is zero. Channel conductivity thus exists at zero voltage between gate and source and is controlled by changing the magnitude and polarity of the gate voltage. A depletion type device is normally-on. For the normally-on

depletion device, a current can flow at a zero gate potential, and the current can be increased or decreased by varying the gate voltage.

Fig. 1 illustrates a neuroelectronic hybrid system, which provides for both electrical (left hand side) and chemical (right hand side) communication.

Referring to Fig. 2, there is illustrated a scheme defining the geometrical relations between a cultured neuron and the transistor gate, and a high seal resistance R_{seal} between the body of the biological solution and the neuron-transistor coupling area. The inventors investigated the parameters that determine the efficiency of the coupling coefficients and recognized the following major obstacles in constructing efficient, reliable and durable neuro-electronic hybrids:

- the formation of the high seal resistance (R_{seal}) between the body of the biological solution and the neuron-transistor coupling area; and
- the continuous mobility and structural plasticity of the neurons relative to the coupling electrodes of the electronic devices.

Seal resistance

The seal resistance depends on the dimensions of the space formed between the transistors floating gate surface and the plasma membrane facing the gate. The geometry and dimensions of this space defines the value of the seal resistance R_{seal} formed between the center of the neuron-gate contact area and the non-contacting region. Theoretical considerations [2-4], as well as experimental considerations show that the larger R_{seal}, the better the electrical coupling. Hence, an electronic device should be designed so as to provide reliably increased R_{seal}.

Continuous mobility and structural plasticity of neurons

A characteristic feature of neurons is their structural plasticity. Under in vivo conditions neurons changes their morphology and connectivity during development, after injury and in relation to various forms of learning and memory processes [5-6]. Likewise, cultured neurons do not maintain a constant position on the device surface [7]. This mobility results in continuous translocation of the cell body, axons and dendrites in respect to the device surface. As a consequence, instability in the shape and amplitude of the recorded potential occur. Additionally,

a large fraction of cultured neurons is often not forming an appropriate physical contact with the sensing device and thus functional contact with the surface electrodes is not established. For a small number of large neurons, this problem might be solved by creating a mechanical "Fence" in the form of a set of polymer pillars that surround the neuron and prevent it from moving away from its location. However, in a multi-transistor array, individual neurons cannot be specifically placed within the "fence", and therefore the electronic device should be designed so as to reduce neuronal translocation.

The technique of the present invention takes an advantage of such known phenomena as phagocytosis, for designing an electronic device (transistor) for communication with living cells. Phagocytosis consists of a process of engulfing foreign particles by cells, and presents a phenomenon of fundamental importance for a large number of cells types and organisms. It is known to be a mean to internalize food (in protozoa), cellular debris (macrophages and neutrophils) or specific signaling molecules. Phagocytosis is defined as the cellular process that leads to the internalization of large particles in the range of up to 0.5μm [8].

Fig. 3A schematically illustrates the cellular mechanisms that underlie phagocytosis. Phagocytosis is an actin dependent process in which extension of the plasma membrane around a particle, or the "sinking" of a particle into the cell leads to its internalization. Accordingly, the process is inhibited by cytochalasin, a toxin that interferes with the polymerization dynamics of actin filaments [9]. Phagocytosis is triggered by the activation of receptors [10] such as Fc-receptors (that mediate the internalization of particles decorated by immunoglobulins), complement-receptors, integrins that mediate the uptake of particles coated with fibronectin [11], mannose receptors that internalize lectins coated particles [12] and others.

As shown in **Fig. 3A**, the main steps leading to the internalization of a particle by phagocytosis are as follows:

Step I: The primary step that triggers phagocytosis is the interaction between the extracellular domains of a receptor molecule and the molecules presented on the target surface. This interaction tethers the target to the membrane (Fig 3A, 1, 2).

Step II: Additional receptors are recruited to the target leading to increased contact between the target and the plasma membrane and initiate the extension of the plasma membrane around the particle (Fig. 3A, 2).

Step III: Signaling from the cytoplasmic domain of the engaged receptors recruit cytoskeletal elements including Arp2/3 that nucleates actin filaments around the particle (Fig. 3A, 3 - the assembly is depicted by the pink coating).

Step IV: Actin filaments together with myosin generate the mechanical force to drive the process of particle engulfment into the cell (Fig. 3A, 4).

Step V: The plasma membrane surrounding the particle is pinched off from the plasma membrane (Fig. 3 A, 5).

The detached plasma membrane that contains the particle is now free to move in the cytosol and fuse with endosoms.

A mechanism utilized in the present invention consists of internalization of the micronail, and provides for better anchor neurons to an artificial surface, as compared to the actual phagocytosis. In phagocytosis, the particle is totally engulfed by the cell, a process that requires the cell to close the membrane over the particle and form a sealed vacuole (which contains the particle – Fig. 3A, 4). As shown in **Fig. 3B**, in contrast to the cellular mechanisms that underlie phagocytosis, the internalization of the micronail requires the cell "swallowing" the head of the micronail, while not "finishing off" the process of vacuole formation (Fig 3B, compare 1 and 2). This difference raises a series of questions related to whether or not the fact that the "pinching off" act is not executed, will interfere with the molecular mechanisms of adhesion

The inventors have found that harnessing the mechanisms of phago/endocytosis provides for anchoring neurons and improving the electrical coupling between the neuron and electronic device (the gate electrode of a transistor). The recognition event between the extracellular domain of the receptor

and the legend that decorate the particle (or the micronail) is the initial and essential event in the cascade that leads to phagocytosis (the tethering of the external particle and the cell). It is known that similar mechanisms and proteins provide also the essential component for the assembly of adhesion junctions. Thus, similar molecular building blocks participate in the attachment of cells to the substrate and to particles that are then phagocytosed. Among the common structural components, just a few are mentioned: talin that binds both cytoskeletal and signaling molecules [13], vinculin and paxillin. This approach is supported by a number of experiments which demonstrate that if a legend mediating phagocytosis is present over a sufficient large substrate area, the cultured cells will attach to the substrate and form adhesion junctions [14].

In spite of the configuration of an unfinished phagocytotic event, the junction formed between the neurons and the micronail is stable. This junction is a functional connection between the transistor and the cell (e.g., neuron) enabling signal transfer in at least one direction, either from the transistor to the cell or from the cell to the transistor through capacitive coupling.

A suitable model for realizing the principles of internalization of the micronails heads is the internalization of beads. The inventors have developed optimal surface chemistry that induce neurons to phagocytose polystyren beads with diameters ranging between 0.2-0.5μm. To this end, the following was carried out: using cultured Aplysia neurons, vertebrate cell lines (that can be induced to differentiate into neurons) and commercially available polystyrene beads with active surface; covalently linking hydrolytic enzymes to the beads thus facilitating degradation of the extracellular matrix and allowing the formation of intimate physical contact between the beads surface and receptor molecules located on the external surface of the plasma membrane; linking molecules that recognize plasma membrane receptors and tether it and facilitate phagocytosis, using adhesion molecules that stabilize the binding of the cell's plasma membrane to the pins base and its surroundings. The results in terms of successful phagocytosis and cell

survival were evaluated by confocal microscope imaging supplemented by electron microscopy.

Figs. 4A-4D illustrate the results (electron micrographs of endocytotic phagocytotic profiles) of another experiment performed by the inventors where the assembly of the sub-membrane coat and endocytosis was induced by exposing cultured *Aplysia* neurons to fragments of other neurons. **Fig. 4A** shows the induction of endocytosis, where "1" denotes an initial stage, noting the darkening of the membrane just beneath the particle on the right hand side of the micrograph, "2" corresponds to the state when plasma membrane begins to invaginate, and "3" notes the increase curvature of the plasma membrane. **Fig. 4B** shows the induction of an endocytotic profile by a particle. It appears as if the cell is sending an extension in support of the engulfment process. **Fig. 4C** shows the endocytotic profile. **Fig. 4D** shows a coated vesicle containing a cell fragment. The dimensions of the profile and coated vesicle is 0.3-0.5μm, the dimensions of the golden head of the micronails

Figs. 5 and 6 illustrate an electronic device (transistor) according to the invention utilizing a gate electrode (poly-silicon floating gate in the present example) formed with micronails, which are protruding from gate electrode surface. **Fig. 5** shows an over view of the micronails configuration on the floating gate, and **Fig. 6** shows the scaled cross-section of the 'nail' structure. As shown, many micronails are provided protruding from a single gate.

The realization of the nails can be done either in 0.18μm or 0.13μm CMOS processes by minor modifications of the commonly used tungsten plugs technology. The following Table I presents values of the different dimensions of the micronail for these two technologies:

CMOS	Dimensions in μm							ASPECT
Technology	A	B	C	D	E	F	G	Ratio
0.18μm	0.805	0.960	0.920	0.290	0.250	0.155	0.40	3.17
0.13μm	0.610	0.740	0.700	0.240	0.20	0.130	0.335	2.91

For the purposes of this experiment, the base (stem-like portion) of micronail *type A* is preferably made of tungsten (but generally from any other suitable electrically conductive material) and is electrically isolated from the solution in which the neurons (or other cell types) are embedded by a layer of silicon nitride of about 20nm in thickness. The top surface of the poly-silicon around the nails, in the plane of the transistor, is isolated from the solution by a layer of silicon nitride of about 40nm. On top of the tungsten base, there is a cap (head portion) of gold or another metal such as for example copper, aluminum, platinum and silver, obtained by standard electroplating or electrolyses plating technique, which is performed on the finished wafers. An alternative process for the formation of the metal cap is by the use of standard lithography.

Fig. 7 shows two types of the Floating Gate Depletion type Transistor (FGDT) based micronails sensors: *Type A* devices are designed to enhance contact with neurons by applying various surface chemistries that facilitate wrapping of the nails by the cell's membrane, and *Type B* devices present a modification of the *type A* devices and are tailored to enhance sensitivity to molecular recognition events and chemical processes, with or without cells presence. Both FGDT types are fabricated using standard CMOS processing steps.

Additional layers of chemically active molecules can be added on top of the nail head to enhance the swallowing of the micronail's head into the neuron, as will be described further below.

A major concern of the micronails structure of the present invention is the influence of the nail on the neuron-transistor electrical coupling by short-circuiting the floating gate (Fig 5). This is relevant only to floating gates connected to micronails that are not engulfed by a neuron.

Generally, two cases are to be considered: In one case, the exposed nail simply shorts the floating gate to the biological solution via the gold head. In the second case, there is an isolating layer over the entire nail surface including the gold head. This insulating layer still increases significantly the capacitive coupling of the floating gate to the ionic solution, consequently reduces the electrical

coupling of the neuron to the floating gate by other nails that are engulfed by the neuron. The problem of coupling can be solved in one of the following ways:

1. By creating a single micronail on each floating gate;

2. By creating a closely packed cluster of micronails on each floating gate, the dimension of this cluster being less than a few microns, which will reduce the chances that one or more of the nails, will not be surrounded by the cell membrane.

The development of bioelectronic enzyme applications [15] requires the immobilization of active proteins onto solid or colloidal substrates such as gold. Proteins adsorb nonspecifically onto clean gold surfaces with denaturation and a reduction of their activity [16-20]. Direct adsorption neither discriminates among protein populations nor controls their orientation, features sometimes desirable for an immobilization protocol. Coverage of the gold surface with alkanethiol self-assembled monolayers (SAMs) [21-23] has made it possible to drastically reduce nonspecific adsorption of proteins [24, 25], to direct the binding of proteins to gold supports, and to control their orientation by using SAMs with ligands complementary to specific binding sites on native proteins [26-32]. Many strategies for protein immobilization on SAMs have been based on previous developments of chromatography supports, which oriented immobilized proteins through charged, hydrophobic, or other affinity group interactions [33-38]. Another known application of SAMs is the use of specific cofactor-apoprotein interactions to assemble enzymes on gold surfaces [39-47]. Also proteins have been modified, by genetic [48-55] or chemical [56-58] procedures, so as to acquire binding sites with affinity for ligands on the monolayer. Electrostatic and hydrophobic [48-49] interactions have also been used in protein immobilization procedures on gold electrodes modified with SAMs of thiols.

The technique of the present invention provides two new approaches applicable to native proteins. The first approach is to introduce programmed lifetime of enzymes via the use of biodegradable linkers, and the second approach

relies on durable aromatic linkers that enhance the electronic coupling of molecular recognition events to the gold (or oxide) containing floating gate (*vide infra*).

Micronails type A

As mentioned above, the *type A* micronails are fabricated from biocompatible metals, for example tungsten rods that are integral part of the gate but insulated from the surrounding by oxide or nitride layers. The bases of the nails are terminated with gold hemispheres (Fig. 7). The molecular decoration of these nails is differentiated according to the various exposed surfaces: gold for the heads and nitride or oxide for the bases. The molecularly modified gold heads present to the cultured cells plasma membrane chemical signals that facilitate its physical uptake by phagocytotic or endocytotic mechanisms (as described above with reference to Figs. 4 and 5). The component of the self-assembled monolayers containing nitride base acts as the cell adhesion interfaces.

Micronails type B

The nails in this case (Fig. 7) are fabricated from poly-silicon rods/bases that are integral part of the poly-Si gate. However, they are insulated from the surrounding by a 20nm thermal-oxide that is grown on top of the poly-Si rod following etching of the nitride layer. In contrast to type *A* devices, here the surface area of the floating gate thermal-oxide coating is maximized to allow better molecular sensing ability and polarization mediated potential sensing.

Surface chemistry-induced internalization of the micronail's heads by plasma membrane invaginations

The technique of the present invention aimed at inducing the internalization of the micronails heads is based on their functionalization by molecules that perform three functions:

(a) Hydrolytic enzymes that facilitate degradation of the extracellular matrix (ECM) and thus allow intimate physical contact between the micronail head surface and receptor molecules located on the external surface of the plasma membrane;

(b) Molecules that recognize plasma membrane receptors and tether them and facilitate phagocytosis or endocytosis of the micronails head, i.e., leading to partial internalization of the micronails by the plasma membrane;

(c) Adhesion molecules that stabilize the binding of the cell's plasma membrane to the nails base and its surroundings.

Hydrolytic Enzymes

Fig. 8 illustrates a process of adhering hydrolytic enzymes to the nails heads surface, showing the micronails' head assembled with phagocytosis signaling peptides (PSP) and hydrolytic enzymes (HE) connected via biodegradable spacer (PLA/PGA). These hydrolytic enzymes include: polysaccharide-degrading enzymes (e.g., sialidase, neuraminidase, hyaluronidases and the like), proteinases including carboxypeptidase and collagenases, and lipid-degrading-enzymes (e.g., lipases and phospholipases).

The lifetime of the hydrolytic enzymes anchored to the gold hemisphere is limited by biodegradability (or "spontaneous" hydrolysis) of the linker chains. The implanted enzymes do not affect the phagocytosis signaling peptides. The biodegradable linker chain is synthesized from poly(lactic-acid) or poly(gluconic-acid) that are non-substrates for the HE used in here. The enzymatic hydrolysis is directed towards saccharides, lipids, and peptides of the ECM that would not attack the lactonic- (or gluconic-) ester bond. In order not to hydrolyze the phagocytosis signaling peptides, carboxypeptidase A is used as the peptide digesting enzyme and the phagocytosis signaling peptides is anchored to the surface through the N-terminal and blocked by t-Bu group at the C-terminal preventing the enzyme recognition and hydrolysis.

Tethering and induction of nail's head internalization

Following the local hydrolysis of the ECM, the recognition of plasma membrane surface molecules by molecules anchored to the gold head of the micronails is expected to internalization of the micronail nevertheless not including the final "pinching off". This event is facilitated by anchoring to the gold head. This

is illustrated in **Fig. 9** showing degradation of the extracellular matrix and partial phagocytosis of the nails by plasma membrane invagination.

Hyperpolarizable molecular transducers can be assembled directly on the nail's Au (*type A*) or SiO_2 (*type B*) exposed area and their head groups anchor plasma membrane surface molecules. Integrins typically recognize short linear amino acid sequences in ECM proteins, one of the most common being Arginine-Glycine-Aspartate (RGD) [65]. Such peptides are grafted onto the gold surfaces via attachment at the N-terminal to surface grafted active ester.

Fig. 10 illustrates peptide anchoring to the base, nitride regions and to the gold nail-heads containing chromophoric layer, in *type A* devices. In case of *type A* devices, chromophores containing surfaces are also grafted with peptides. In the case of *type B* devices, chromophores containing surfaces are assembled on SiO_2 surfaces by treating the surface with the appropriate coupling agent (e.g., 3-bromopropyl(trichlorosilane)), and thus the device will couple alkylhalide functionality to the oxide in step *i*, followed by steps *ii-iv* as shown in the example of RGD coupling of Fig. 10.

The second approach according to the invention for creating intimate neuron-nail contacts relies on recognition sites of hyaluronan, the polysaccharide part of proteoglycan in the extracellular matrix. The glucosaminoglycan repeating unit of hyaluronan or sialic acid can be easily coupled via siloxane linkages of the sugar's hydroxyl-group. This coupling can be conducted directly on the nitride surface and via chromophoric layer self-assembled on the gold-heads in *type A* devices and on the oxide rods in *type B* devices. This is illustrated in **Fig. 11**, showing saccharides anchoring to the nitride regions and to the gold nail-heads containing chromophoric layer, in *type A* devices. *Type B* devices will couple alkylhalide functionality to the oxide in step *i*, followed by steps *ii-iv*. Hyaluronic acid and Sialic acid coupling is given as a non-limiting example.

Adhesion of the cell to the substrate

The inventors have developed the 2D-PAN, a positively charged monolayer of polyaniline, to adhere the plasma membrane to the micronail stem and the active

planes of the transistor (e.g., FGDT). The main steps of this method are illustrated in **Fig. 12**. The method relies on the electrostatic assembly of a monolayer of monomers (anilinium-ions) followed by their polymerization (to polyaniline, PAN). Polyanion (polystyrenesulfonate sodium salt, PSS) deposition has led to rougher surfaces than the small-molecules containing template layer [51]. The electrostatic adhesion of the positively charged anilinium monomers is followed by chemical oxidation with ammonium-peroxydisulfate or enzymatically with horseradish-peroxidase (HRP) to yield the 2D-PAN (co-pending U.S. application No. 60/454,613 assigned to the same assignee). The use of PSS template layer for 2D-PAN self-assembly has created a special interface for neurons growth. The combination of positively charged (quaternary amines) and rough interface has led to very strong cell adhesion to the micronail.

The advantages of this layer over the standard neurophilic layers (*e.g.*, laminine, poly-D-lysine, and fibronectin.) are associated with their diminished vertical dimensionality and reduced electrical resistively. A better capacitive coupling between neurons and MOS-devices is provided when the neurons are placed closer to the sensing area (2-4 nm *vs.* few hundreds) and where the membrane potential is less shielded (conducting polymer *vs.* insulating layers). Thus, the coupling of the neurons to the nitride- and oxide-containing surfaces of the device should add to the sensitivity of both device types.

The assembly of 2D-PAN to gold surfaces has been developed [52] and can be straight-forward implemented to the micronail Au-head. The inventors have studied the warping of Au-nanoparticles (NP) with PAN-monolayers, and investigated their interface with *Aplysia* neurons. The adherence of Aplysia neuron to self-assembled PAN monolayer on an electrostatically bound Au-NP to positively charged glass substrate is illustrated in **Fig. 13** - an electron microscopy image of the interface between Aplysia's plasma membrane and a monolayer of 5nm Au particles self-assembled with 2D-PAN monolayer on a glass substrate. The tight junction (of about 10-30 nm) is indicative of the decrease in R_{seal} and the enhanced sensitivity of the micronail containing FGDT based neurosensors.

Molecular recognition micronail-heads

The chemical anchoring of receptors and enzymes can be conducted by covalent linkages to the gold nail binding by short and rigid coupling agent. **Fig. 14** illustrates an example of a short and rigid molecular coupling of receptor or enzyme to the gold head by first assembling *p*-aminothiophenol followed by condensing cyanuric acid allowing coupling of the receptor/enzyme through amine condensation. In the present example, neurotransmitter site is shown containing proteins (enzyme/receptor; E/R) anchoring to gold surfaces via rigid and short spacer (*type A*). The assembly to oxide surfaces is conducted similarly excluding step *i* (*type B*), as disclosed in co-pending IL application No. 152246 assigned to the assignee of the present application.

As indicated above, the formation of chemical synapse composed of a neuronal presynaptic element and an electronic device that serves, as a post-synaptic element, enables to link neurons to the electronic device not only by electrical signals but also by released neurotransmitters. Such chemical linkage opens up a novel way to link the nervous system with the electronic world and allows simulating the natural way by which neurons as well as neurons and muscles communicate with each other. It should be emphasized in this respect the unidirectional communication between excitable cells is mainly executed by chemical synapses.

The inventors have developed a highly sensitive (10^{-8} M) sensor for acetylcholine and conducted experiments in which polystyrene beads coated with bioactive molecules were shown to induce the ultrastructural differentiation of presynaptic terminal. The micronails surface can be coated with a variety of signaling molecules (as described above) and receptor molecules that recognize and binds acetylcholine, glutamate, GABA, serotonin and others.

CLAIMS:

1. An electronic sensor device comprising a transistor structure, in which a gate electrode is formed with at least one micronail protruding from the surface thereof.

2. The device of Claim 1, wherein the micronail is a rod that is an integral part of the gate electrode and is insulated from surroundings.

3. The device of Claim 2, wherein the micronail is a poly-silicon rod, which is integral part of the poly-silicon gate electrode, and is insulated from the surrounding by a thermal-oxide layer.

4. The device of Claim 3, wherein said thermal-oxide layer has a thickness of about 20nm.

5. The device of Claim 3, wherein said thermal-oxide layer is grown on top of the poly-silicon rod following etching of a nitrate layer.

6. The device of Claim 3, wherein the thermal-oxide coating of the gate electrode is maximized to improve molecular sensing ability and polarization mediated potential sensing.

7. The device of Claim 1 for communication with a living cell, wherein said micronail has a base portion and a head portion, said head portion being a metal cap, thereby facilitating wrapping of the nail by a membrane of the cell.

8. The device of Claim 7, wherein the base portion of the micronail is made of tungsten and is electrically isolated from a solution, in which the cell is embedded, by a layer of silicon nitride.

9. A micronail structure having a head region and a base region, wherein said head region carries hydrolytic enzymes that facilitate degradation of the extracellular matrix.

10. The micronail structure according to claim 9, wherein said head region further carries molecules that recognize plasma membrane receptors located on the external surface of the plasma membrane.

11. The micronail structure of claim 9, adapted to carry adhesion molecules that stabilize the binding of the cell's plasma membrane to the nails' base and its surroundings.

12. The micronail structure of claim 9 wherein said base is made of an electrically conductive material and is electrically isolated from the surrounding environment, and said head is made of a metal selected from gold, copper, aluminum, platinum and silver.

13. The micronail structure of claim 12 wherein said base is made of tungsten, and is electrically isolated from the surrounding environment by a layer of silicon nitride.

14. The micronail structure of claim 9, fabricated from poly-silicon material and being electrically insulated from the surrounding by an oxide layer that is grown on top of the poly-silicon material.

15. The micronail of anyone of claims 9, formed on top of a gate electrode of a transistor structure.

16. The micronail structure of claim 9, wherein said hydrolytic enzyme is selected from polysaccharide-degrading enzymes, proteinases and lipid-degrading-enzymes.

17. The micronail structure of claim 9 wherein said hydrolytic enzyme is connected to said head region through a biodegradable spacer molecule.

18. The micronail structure of anyone of claims 9, wherein said head region carries recognizing molecules that recognize plasma membrane receptors, where the recognizing molecules are integrins that recognize short linear amino acid sequences in ECM proteins.

19. The micronail structure of claim 9, wherein said head region carries recognizing molecules that recognize polysaccharides that are part of proteoglycans in the ECM plasma membrane.

20. The micronail structure of claim 11, wherein said adhesion molecules are in the form of a charged monolayer.

21. The micronail structure of claim 19, wherein said charged monolayer is a positively charged monolayer of polyaniline.

22. The micronail structure of claim 20, wherein said positively charged monolayer of polyaniline is assembled on a polystyrenesulfonate layer, said polystyrenesulfonate layer comprising anion units connected through a linking moiety to the micronail.

23. An electronic sensor device comprising a transistor structure, in which a gate electrode is formed with at least one micronail protruding from the surface thereof, said micronail having a structure of claim 9.

24. An electronic sensor device according to claim 1 adapted for sensing an agent selected from acetylcholine, glutamate GABA and serotonin.

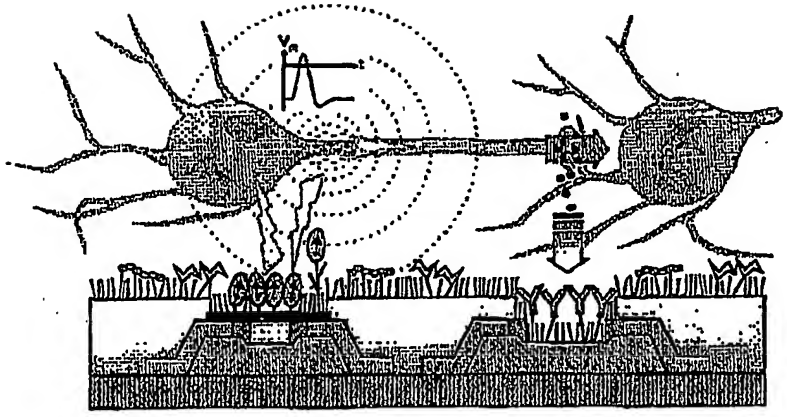
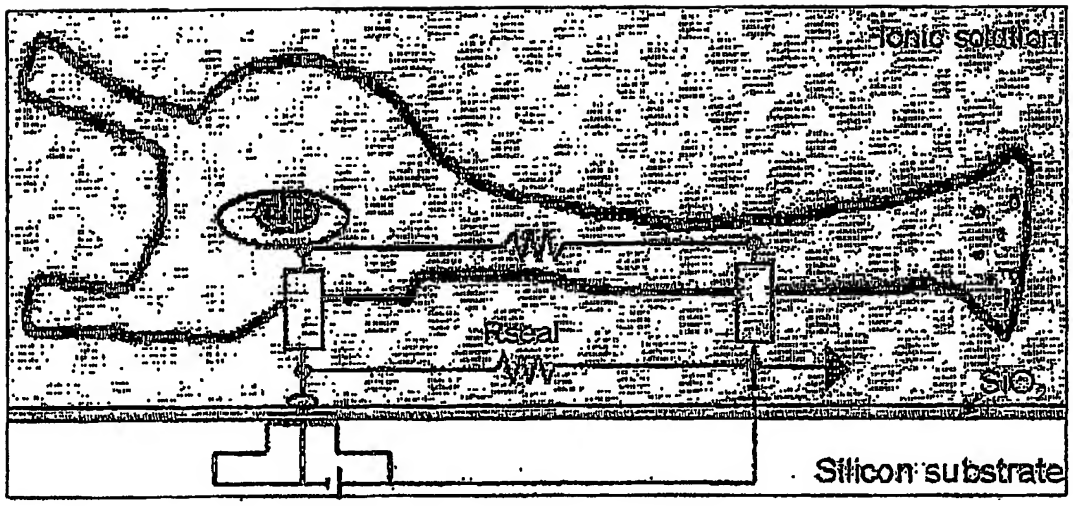


Fig. 1



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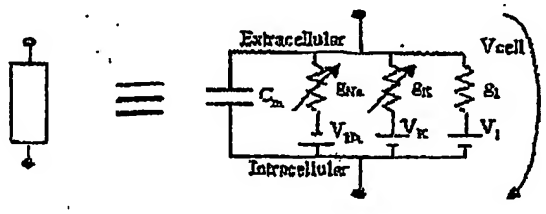


Fig. 2

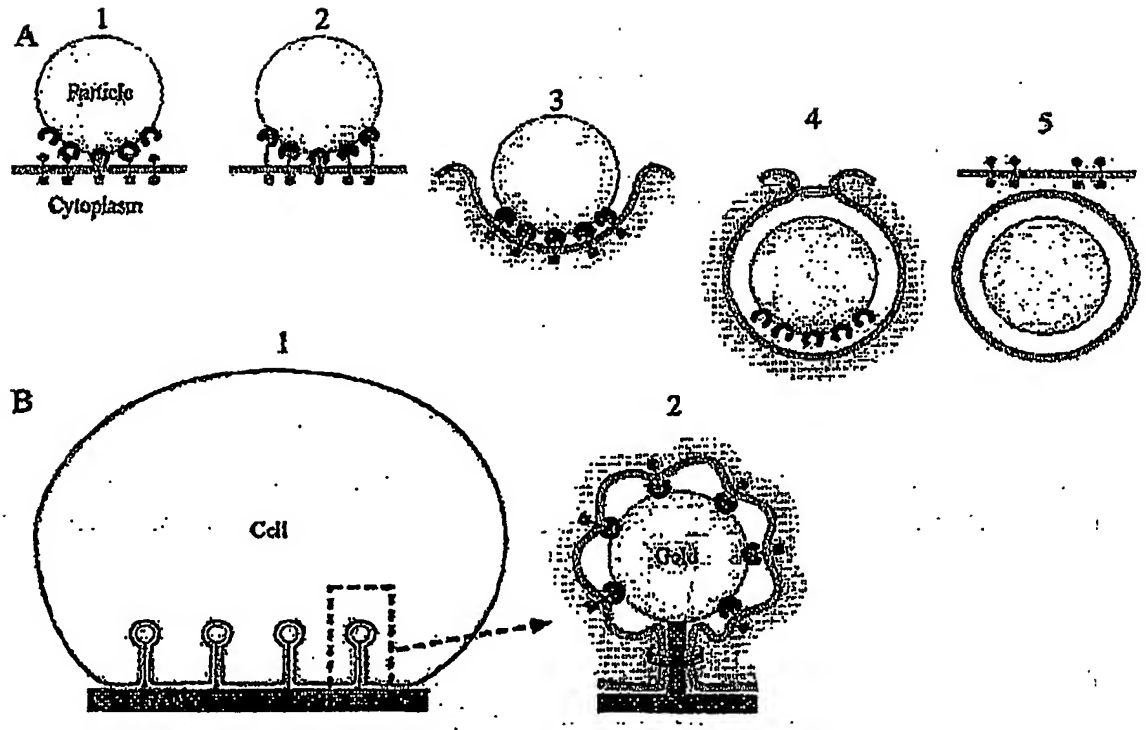


Fig. 3

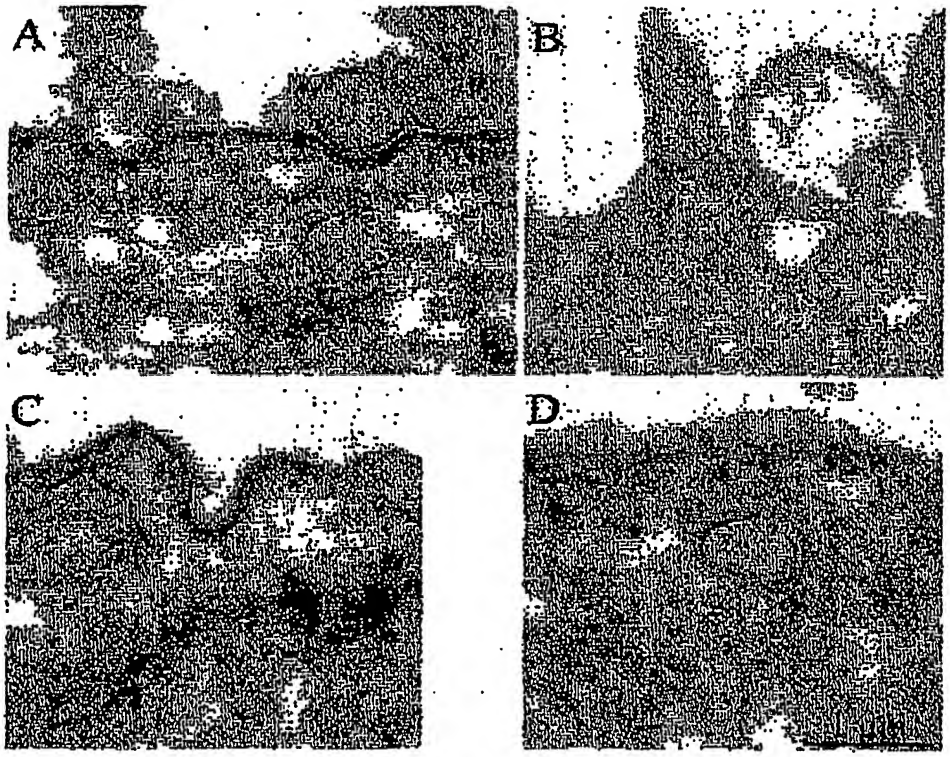
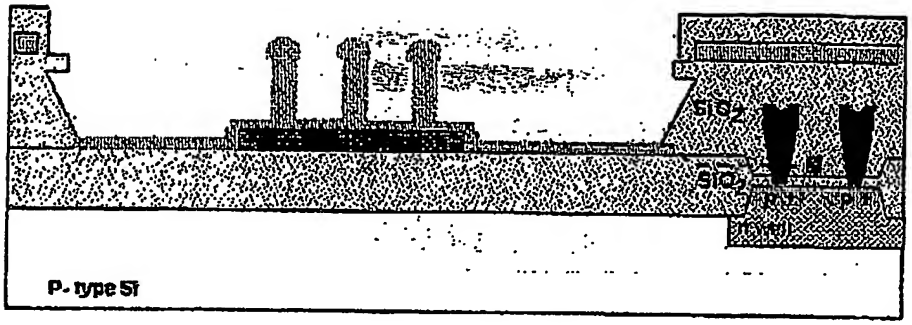


Fig. 4


SiO2
SiO2
P-type Si

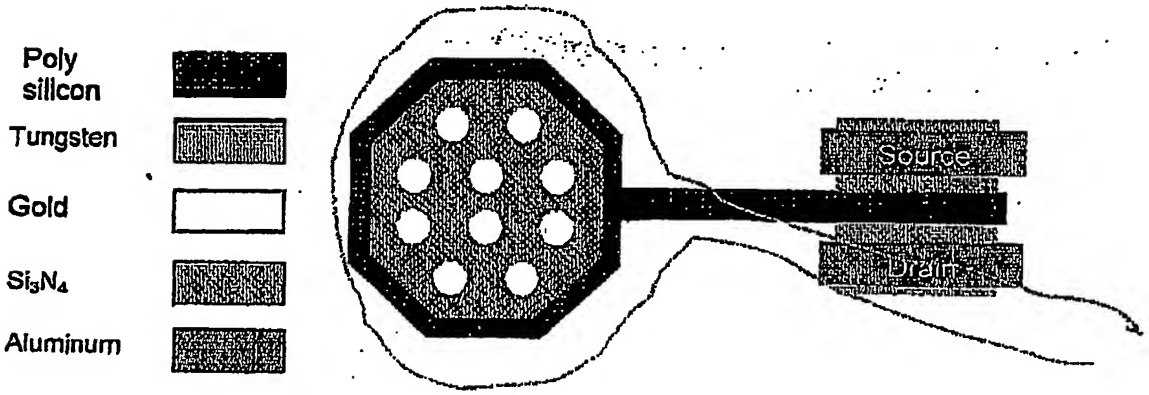

Poly silicon
Tungsten
Gold
Si3N4
Aluminum
Source
Drain

Fig. 5

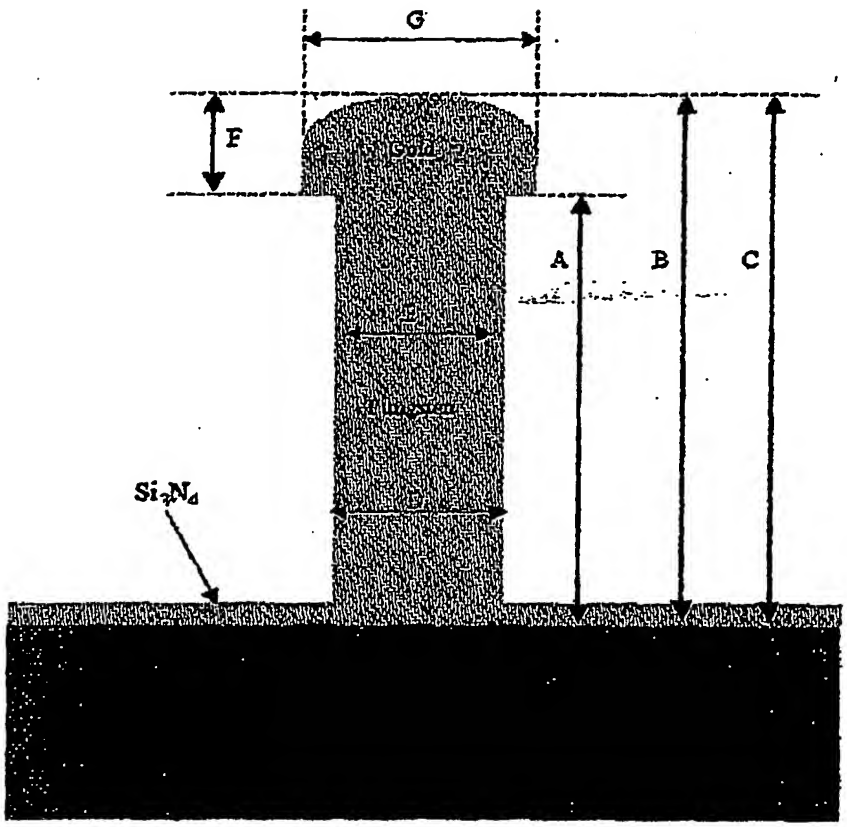


Fig. 6

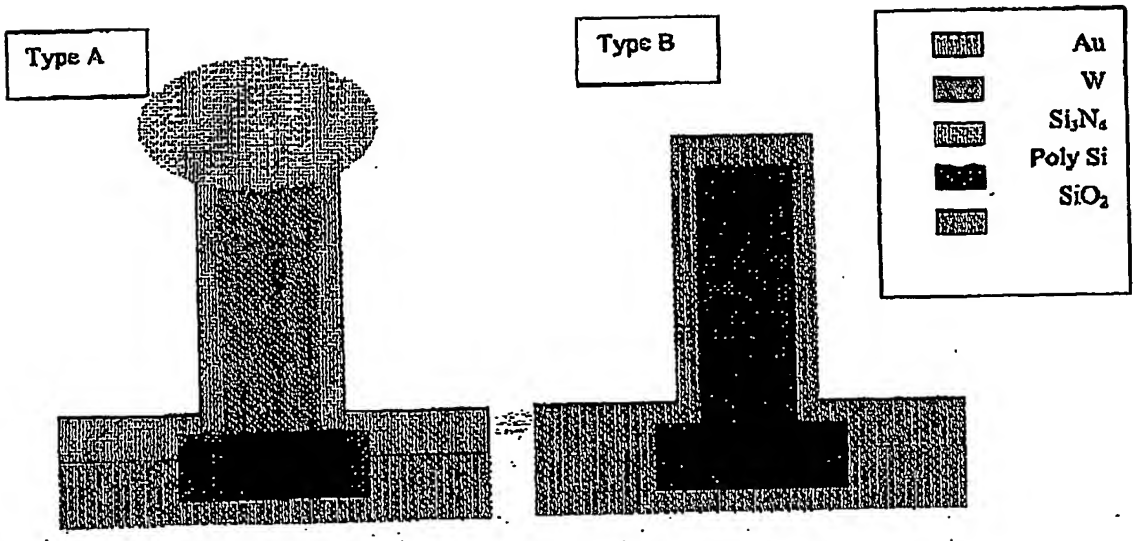


Fig 7

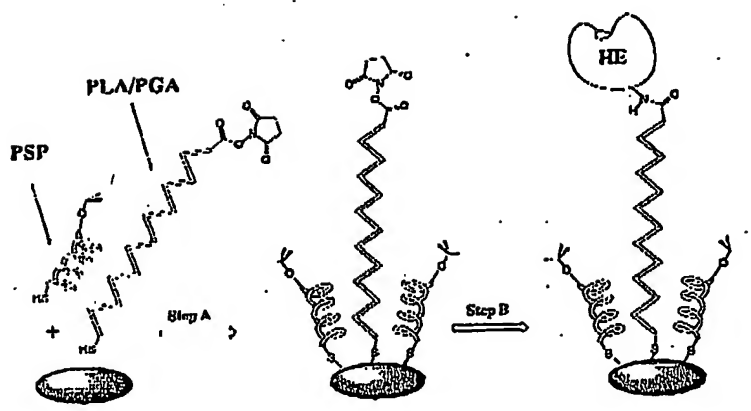

PLA/PGA
PSP
HE
Step A
Step B

Fig. 8

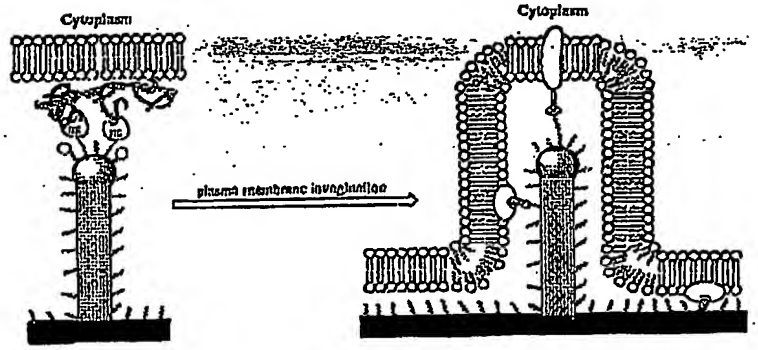

Cytoplasm
Cytoplasm
plasma membrane invagination

Fig. 9

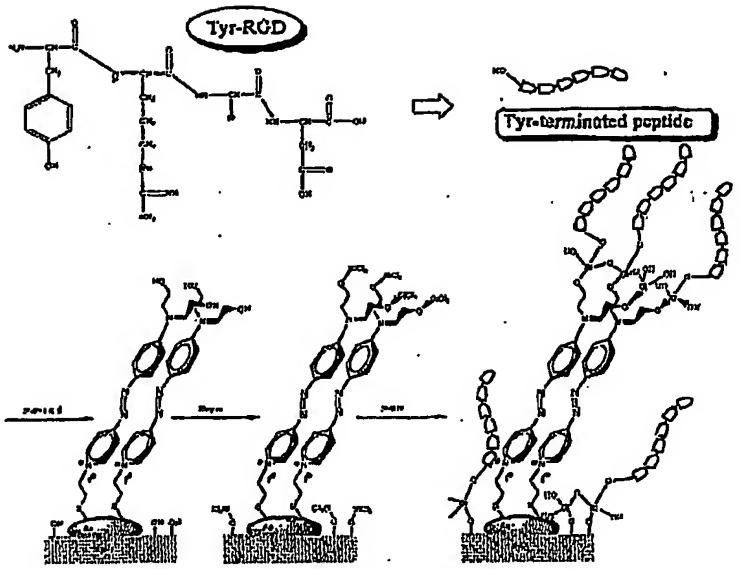


Fig. 10

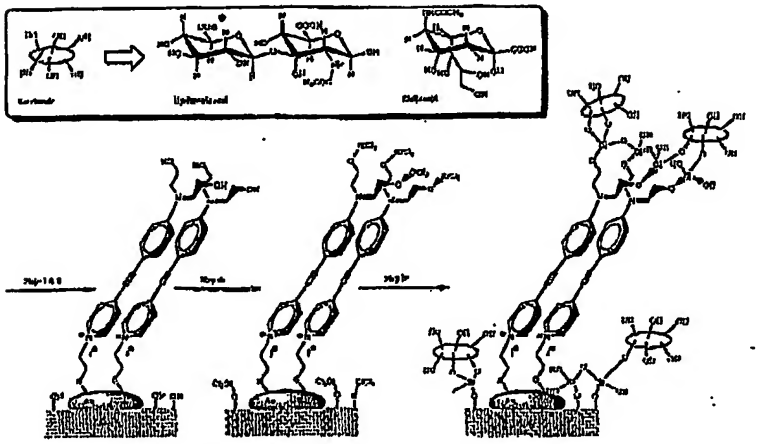


Fig. 11

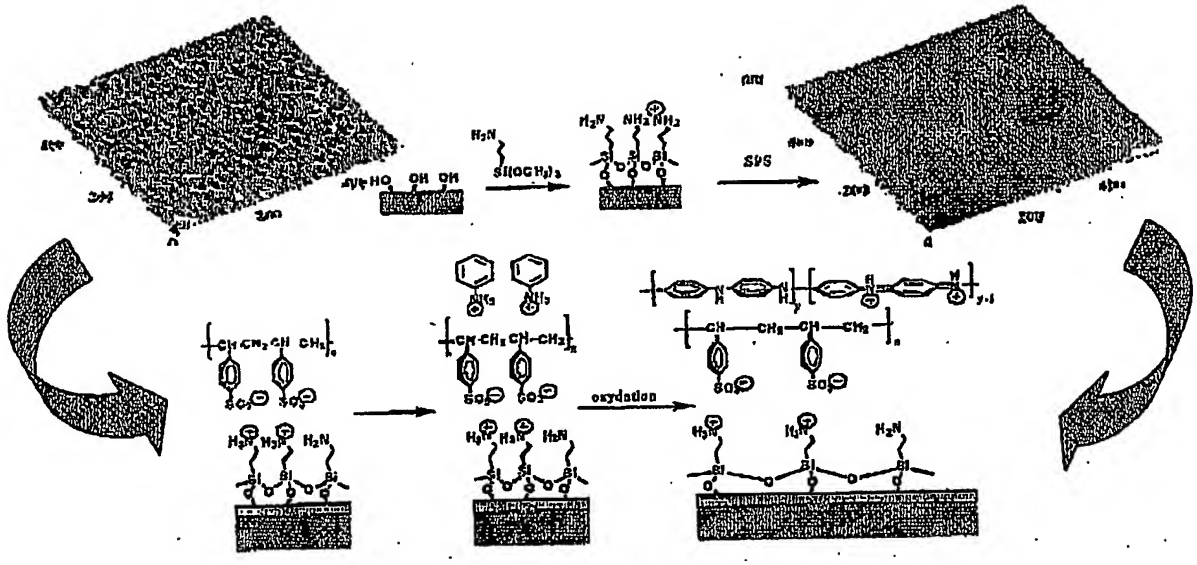


Fig. 12

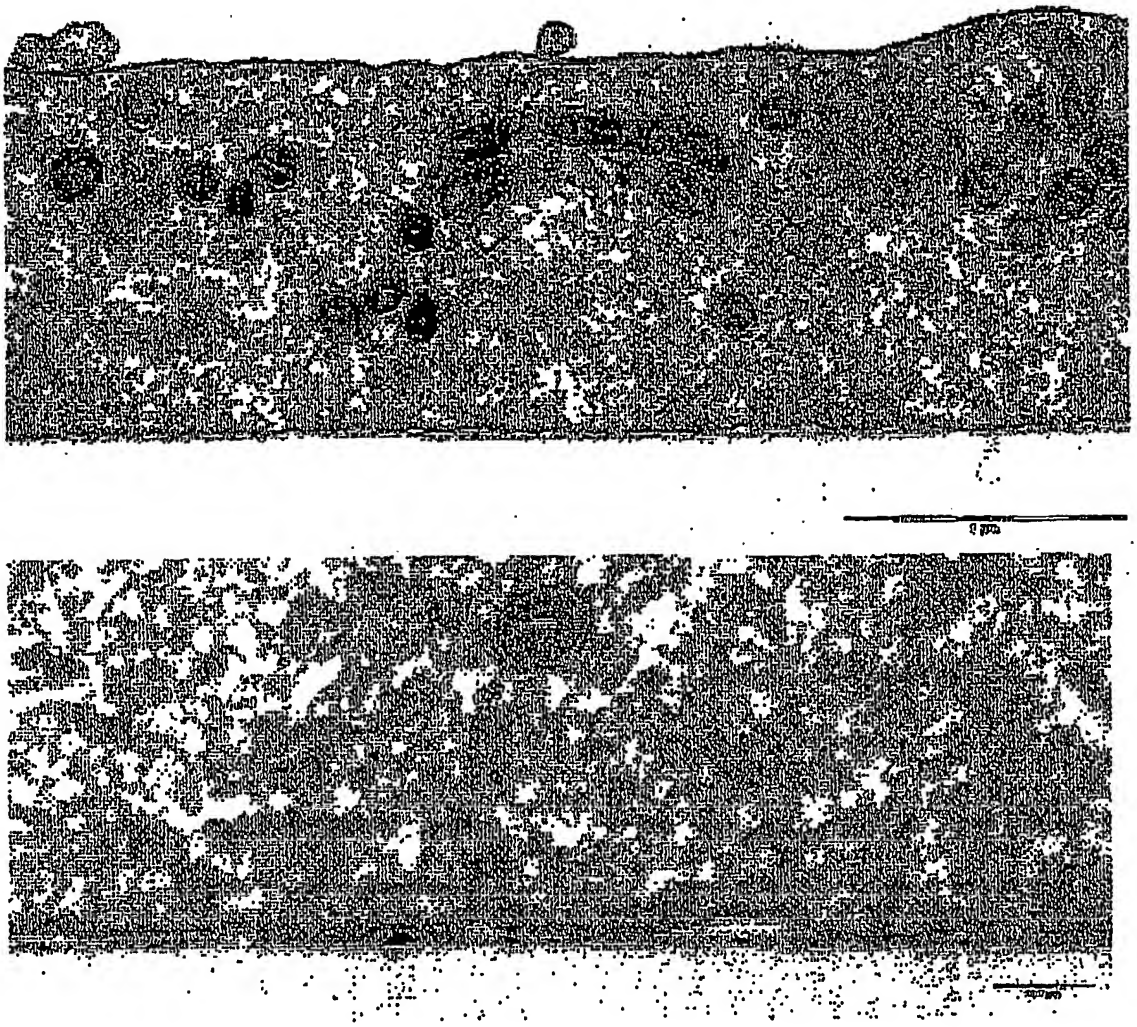


Fig. 13

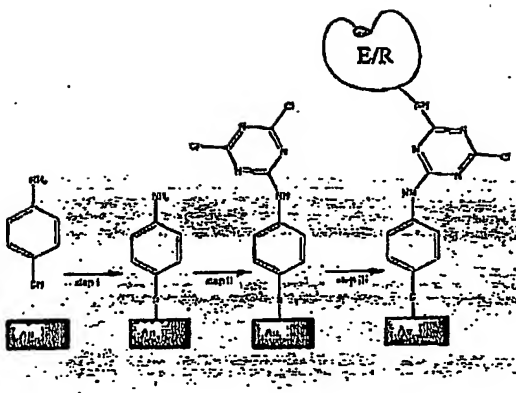


Fig. 14